Kidney International, Vol. 63 (2003), pp. 1256–1264

Activation of the ERK pathway precedes tubular proliferation in the obstructed rat kidney

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Activation of the ERK pathway precedes tubular proliferation in the obstructed rat kidney.

Background. In vitro studies suggest that activation of the extracellular signal-regulated kinase (ERK) pathway plays a critical role in the proliferation of tubular epithelial and myofibroblast-like cells. However, little is known of ERK activation in individual cell types in normal or diseased kidney. The aims of this study were to (*1*) localize ERK activation within the kidney, and (*2*) examine the relationship between ERK activation and cell proliferation in the injured kidney.

Methods. Unilateral ureteric obstruction (UUO) was induced in groups of six Wistar rats, which were killed at 30 minutes, 6 hours, and 1, 4, or 7 days after obstruction. Activation of ERK was identified using antibodies specific for the phosphorylated form of ERK (pERK) in Western blots and immunostaining. Proliferating cells were detected using bromodeoxyuridine (BrdU).

Results. Western blotting showed abundant expression of the two ERK isoforms, ERK-1 and ERK-2, in normal rat kidney. Low levels of activated ERK (pERK-2 > pERK-1) were detected in normal rat kidney by Western blotting. Immunostaining showed that ERK activation in normal kidney was largely restricted to collecting ducts in the outer medulla. Within 30 minutes of ureter obstruction, Western blotting showed a six-fold increase in ERK activation followed by a second peak (14-fold increase) on days 4 and 7. The initial peak of ERK activation was localized to medullary collecting ducts and the thick ascending limb of Henle (TALH), whereas the second peak corresponded to a progressive increase in ERK activation in dilated collecting ducts and in interstitial cells in the cortex. Proliferation of tubular epithelial cells closely followed the pattern of ERK activation, being evident first in medullary collecting ducts and TALH on day 1, and then in cortical collecting ducts from day 4.

Conclusion. This study has identified a discrete pattern of ERK activation in normal rat kidney and an increase in ERK activation following obstruction. The temporal and spatial relationship in which ERK activation preceded tubular cell proliferation suggest that ERK signaling plays a key role in tubular epithelial cell proliferation in the injured kidney.

Key words: ERK, MAPK, p42/44, tubule, epithelial, proliferation, myofibroblast, macrophage.

Received for publication June 9, 2002
and in revised form September 13, 2002
Accepted for publication November 21, 2002

Activation of the extracellular signal-regulated kinase (ERK) pathway plays an important role in many physiologic processes, such as growth factor–induced cell proliferation [1, 2]. Following binding of ligands, such as epidermal growth factor and angiotensin II, to their receptors on the cell surface, a cascade of phosphorylation events leads to dual phosphorylation of the Thr-Glu-Try motif in the two isoforms of ERK (ERK-1 and ERK-2, also known as p44 and p42, respectively). Once dual phosphorylated, ERK becomes an active kinase and can migrate to the nucleus where it phosphorylates specific transcription factors, leading to transcription of specific genes, such as cyclin D1, which plays a key role in the induction of the cell cycle [1, 2].

Most of our understanding of the role of ERK in renal cell responses comes from in vitro studies using drugs that block the function of the mitogen activated protein kinase/ERK-kinase (MEK-1) responsible for ERK activation. For example, ERK activation plays a crucial role in the response of cultured mesangial cells to a variety of stimuli, such as epidermal growth factor, angiotensin II, high glucose, and mechanical stretch [3–6]. However, it is difficult to relate these studies to the normal or diseased kidney since we know very little about ERK activation in situ. A number of studies using detergent extracts of the kidney have demonstrated an increase in ERK activation in diseased states [5, 7, 8]. However, knowledge of the cell types in which ERK activation occurs in the normal and injured kidney would be a significant advance in our understanding of the role of this pathway in the kidney.

The aims of this study were to identify the cell types in which ERK activation occurs in normal rat kidney and to examine how this is changed in a pathologic state. We examined the obstructed kidney because this model

features mechanical stretch and a marked proliferative response of tubular epithelial cells and fibroblast-like cells [9], features that are associated with ERK activation in renal cell types in vitro.

METHODS

Unilateral ureteric obstruction

Female Wistar rats (230 to 250 g) were obtained from Monash Animal Services, Melbourne, Australia. Animals were anesthetized with ketamine chloride and underwent an abdominal midline incision and the left ureter was located. Two ties were made using 4.0 silk and the ureter was cut between the ties to avoid retrograde urinary tract infection. Groups of six rats were killed at 30 minutes, 6 hours, and 1, 4, or 7 days after unilateral ureter ligation. A group of normal Wistar rats was also examined. To assess cell proliferation, animals were given bromodeoxyuridine (BrdU) (50 mg/kg intraperitoneally) 3 hours before being killed.

Antibodies and growth factors

Mouse monoclonal antibodies (mAb) used were E10, antiphosphorylated ERK-1,2 (Cell Signaling Technology, Inc., Beverly, MA, USA); 1A4, anti-α-smooth muscle actin (α-SMA) (Sigma-Aldrich, Castle Hill, NSW, Australia); ED-1, anti-CD68, which labels monocytes and macrophages (Serotec, Oxford, UK); and M744, anti-BrdU (Dako, Glostrup, Denmark). Polyclonal antibodies (pAb) used were rabbit antiphosphorylated ERK1,2 (Cell Signaling Technology, Inc.); K-23, a rabbit antibody recognizing ERK-2 and, to a lesser extent, ERK-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); rabbit antiaquaporin 2 (Calbiochem, San Diego, CA, USA) was used as a marker of collecting ducts, and sheep anti-Tamm-Horsfall protein [10] was used as a marker of the thick ascending limb of Henle (TALH) and distal convoluted tubules. Secondary polyclonal antibodies used were goat antimouse immunoglobulin (IgG) conjugated with either horseradish peroxidase (HRP) or alkaline phosphatase (AP) (Dako); rabbit antigoat IgG conjugated with HRP (Dako); goat antirabbit IgG conjugated with HRP (Silenus Laboratories, Melbourne, Australia); complexes of HRP-conjugated mouse, goat, or rabbit anti-HRP IgG (PAP); and complexes of AP-conjugated mouse anti-AP IgG (APAAP) (Dako).

Western blotting

One half a kidney was homogenized on ice in 2 mL lysis buffer (10 mmol/L Tris-HCl, pH 7.4, 100 mmol/L NaCl, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 mmol/L ethyleneglycol tetraacetate (EGTA), 1 mmol/L NaF, 2 mmol/L Na_2VO_4, 1% Triton X-100, 10% glycerol, 0.5% deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 10% protease inhibitor cocktail (Sigma-Aldrich), left on ice for 10 minutes with regular vortexing, and tissue debris removed by centrifugation at 15,000 × *g* for 20 minutes and the supernatant aliquoted and stored at –80°C. Lysates (15 ug per lane) were run on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidine difluoride (PVDF) membranes (Millipore, Bedford, MA, USA) by electroblotting in 25 mmol/L Tris-HCl, pH 8.5, 192 mmol/L glycine, 20% methanol overnight using a Bio-Rad Transblot apparatus (Bio-Rad Laboratories, Hercules, CA, USA). Blots were blocked for 2 hours in 5% nonfat milk powder in 20 mmol/L Tris-HCl, pH 7.6, 137 mmol/L NaCl, and 0.05% Tween 20 and washed 5 times in 20 mmol/L Tris-HCl, pH 7.6, 137 mmol/L NaCl, and 0.05% Tween 20. Blots were then incubated overnight at 4°C with rabbit anti-pERK antibody in the blocking solution plus 5% bovine serum albumin (BSA), washed, and then incubated for 1 hour with HRP-conjugated sheep antirabbit IgG in binding buffer. After washing, bound antibody was detected using SuperSignal® chemiluminescent substrate (Pierce, Rockford, IL, USA). Chemiluminescent emissions were captured on Kodak XAR film. Blots were stripped and reprobed using the rabbit anti-ERK-2 antibody. Densitometric analysis used the Gel-Pro Analyzer program (Media Cybernetics, Silver Springs, MD, USA).

Immunohistochemistry

Immunohistochemistry staining was performed on tissues fixed in 4% formalin and embedded in paraffin, or staining with the ED-1 mAb-used cryostat sections of tissues fixed in 2% paraformaldhyde-lysine-periodate, as previously described [11]. Tissue sections were placed in 0.01 mol/L citrate buffer, pH 6.0, and heated for 10 minutes in a microwave oven. This treatment was used in detection of pERK-1,2 (E10), BrdU (M744), ERK-1,2 (K-23), and macrophages (ED-1), but was not used in detection of α-SMA or Tamm-Horsfall protein. Sections were blocked in 10% fetal calf serum (FCS) and 10% normal sheep serum in phosphate-buffered saline (PBS) for 30 minutes, and then in 5% BSA in PBS for 30 minutes each and then incubated with the primary antibody in 10% normal rat serum and 1% BSA overnight at 4°C. After washing, endogenous peroxidase was inhibited by incubation in 0.3% H_2O_2 in methanol for 20 minutes. Next, sections were incubated sequentially with HRP-conjugated goat antimouse or sheep antirabbit IgG followed by mouse or rabbit PAP for 45 minutes each, and developed with diaminobenzidine (Sigma-Aldrich) to give a brown color or with Vector SG (Vector Laboratories, Burlingame, CA, USA) to give a blue/gray color (Tamm-Horsfall protein and α-SMA staining). Some sections were counterstained with periodic acid-Schiff (PAS) reagent minus hematoxylin.

Those sections being double labeled were next micro-

wave treated to prevent antibody cross reactivity [11], blocked as above, and then incubated with primary antibody overnight at 4°C. After washing, sections were incubated with AP-conjugated secondary antibodies and APAAP and developed with Fast Blue [aquaporin-2 (AQP-2), ED-1]. Alternatively, primary antibodies were detected using HRP-conjugated secondary antibodies and PAP and developed with diaminobenzidine (pERK-1,2).

Specificity of the immunohistochemistry staining was demonstrated in two ways. First, omission of the primary antibody or the use of an isotype-irrelevant control antibody gave no staining. Second, incubation of antibodies with the immunizing peptide (phospho-ERK peptide or ERK-2 peptide) completely blocked the respective immunostaining signal.

Quantification of immunohistochemistry staining

Immunostaining for pERK, BrdU, ED-1 (with PAS counterstain) was quantified as follows. Predetermined higher power fields (×400) of the cortex (12 fields, avoiding glomeruli) and the outer medulla (9 fields) were scored using an eye-piece graticule for the number of pERK- or BrdU-stained tubular epithelial cells and the number of pERK-, BrdU-, or ED-1–stained interstitial cells expressed as cells per mm^2. The area of α-SMA immunostaining was assessed in predetermined low power fields (×100) of the cortex (3 fields) and the outer medulla (2 fields) which were captured by a digital camera and the area stained determined using Image Pro Plus software (Media Cybernetics).

Statistical analysis

Statistical differences were analyzed by the one-way ANOVA (analysis of variance) using the Dunnett multiple comparison test on GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Data was recorded as the mean ± standard deviation (SD) and $P < 0.05$ was considered significant.

RESULTS

ERK activation in normal rat kidney

Western blotting shows constitutive expression of ERK-1 and ERK-2 isoforms in lysates from whole rat kidney (Fig. 1). Immunohistochemistry showed diffuse staining for ERK-1,2 in all tubular segments, except the thin limb of Henle, whereas glomeruli were negative (data not shown).

Activation of ERK was identified using an antibody specific for the dual-phosphorylated forms of ERK-1 and ERK-2. Western blotting identified pERK-2, and to a lesser extent pERK-1, in lysates of whole kidney (Fig. 1). Immunohistochemistry staining identified a discrete pattern of tubular ERK activation in normal rat kidney, being most prominent in the outer medulla (Fig. 2a). Double immunostaining using AQP-2 demonstrated that

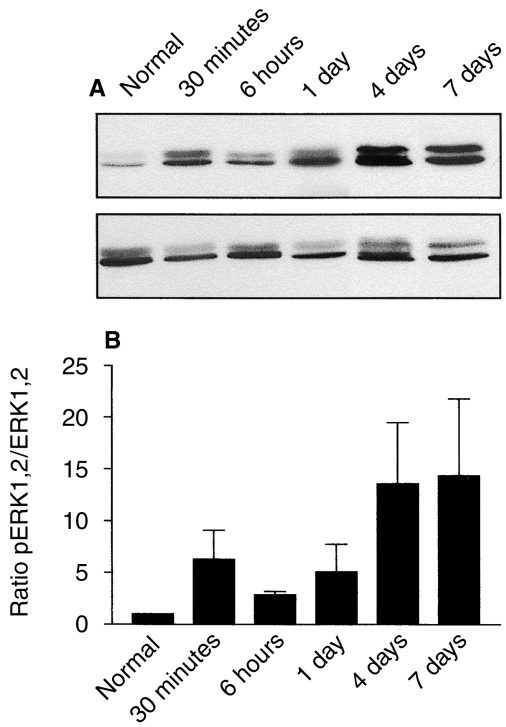

Fig. 1. Extracellular signal-related kinase (ERK) activation in lysates of normal and obstructed rat kidney. Protein extracted from a half kidney from normal rat kidney or at different times after unilateral ureteric obstruction (UUO) was examined by Western blotting. (*A*) The upper panel was probed with an antibody recognizing the phosphorylated (activated) forms of ERK-1 (pERK-1, upper band) and ERK-2 (pERK-2, lower band). The lower panel shows the blot reprobed with an antibody recognizing both phosphorylated and nonphosphorylated forms (i.e., total) ERK-1 (upper band) and ERK-2 (lower band). (*B*) Densitometric analysis presented as the ratio of activated ERK to total ERK (pERK/ERK).

ERK activation is restricted to collecting ducts (Fig. 3a). Both AQP-2 and ERK activation were restricted to the principal cells of the collecting duct (Fig. 3a). ERK activation was not uniform along the collecting duct, being absent in the inner medulla and present in only occasional cells in the cortical collecting duct (Fig. 3b). Of note, pERK was present in both the nucleus and cytoplasm of tubular epithelial cells (Figs. 2 and 3). No activation of ERK was apparent in glomeruli (Fig. 3b), whereas ERK activation was seen in vascular smooth muscle cells (Fig. 3b). The specificity of pERK-1,2 immunostaining was demonstrated by the ability of an ERK phosphopeptide to block the staining (Fig. 2c).

ERK activation in the obstructed kidney

Unilateral ureteric obstruction (UUO) results in fluid retention within the kidney, causing rapid swelling which is evident as early as 30 minutes postobstruction. Tubular dilation occurs rapidly, which is most prominent in the collecting ducts. This is followed by tubular proliferation, interstitial accumulation of macrophages and myofibroblasts, and interstitial fibrosis.

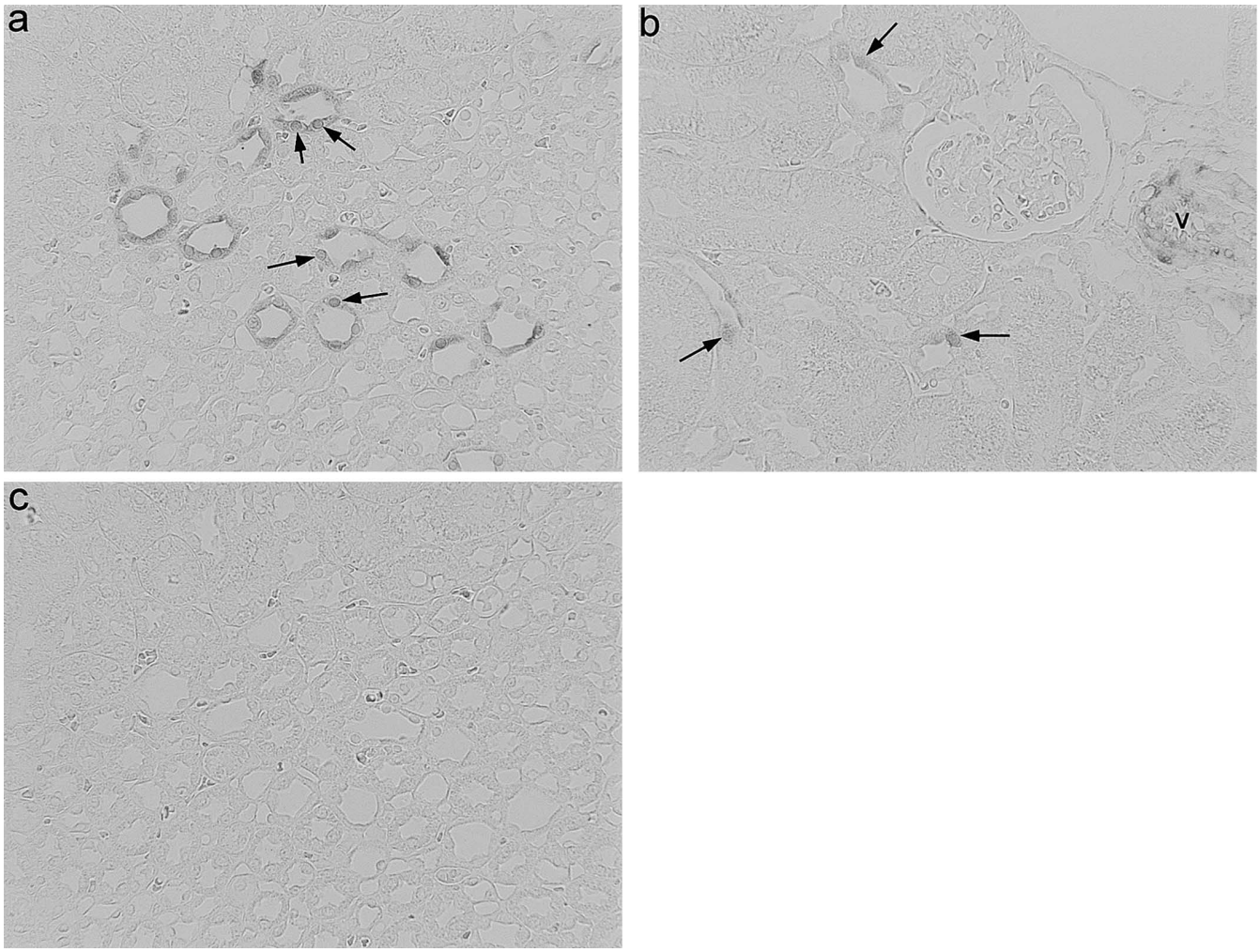

Fig. 2. Extracellular signal-related kinase (ERK) activation in normal rat kidney. (*a*) Normal rat kidney shows immunostaining for phosphorylated ERK (pERK) in the collecting ducts of the outer medulla. Staining is evident in both nuclei (arrows) and cytoplasm. (*b*) Occasional collecting ducts within the cortex exhibit pERK immunostaining, which is both nuclear (arrows) and cytoplasmic. Staining of smooth muscle cells (SMA) in a vessel wall (v) is also seen. No pERK staining is seen within the glomerular tuft. (*c*) Section serial to (a) in which staining is abolished by incubation of pERK antibody with the phospho-ERK peptide antigen. Magnification, ×400.

Western blotting showed no change in renal expression of ERK-1,2 during the 7-day time course of UUO (Fig. 1). In contrast, there was a sixfold increase in ERK activation within 30 minutes of ureter obstruction. There was a partial reduction in ERK activation at 6 hours and then a second peak of ERK activation (14-fold increase) was seen on days 4 and 7 (Fig. 1). Immunostaining showed that the dramatic increase in ERK activation at 30 minutes after UUO was due to ERK activation in collecting ducts and in the TALH in the outer medulla (Fig. 3c). Quantification of immunostaining showed a dramatic increase in the number of medullary tubular epithelial cells showing ERK activation 30 minutes post-UUO, which was followed by a gradual decline over the 7-day time course (Fig. 4a).

In contrast to the medulla, ERK activation was unchanged in cortical tubules at 30 minutes after UUO, but increased progressively thereafter (Fig. 4b). The increase in ERK activation in the cortex was largely limited to collecting ducts, many of which were dilated, and interstitial cells (Fig. 3 e and f).

ERK activation precedes tubular proliferation in the obstructed kidney

Cell proliferation was assessed by BrdU incorporation. Tubular proliferation followed a pattern similar to that seen for ERK activation. The initial tubular proliferative response was seen at day 1 in the outer medulla, being localized mainly in dilated collecting ducts and, to a lesser extent, in TALH (Figs. 3d and 4A). Proliferation of cortical tubules became prominent on day 4 post-UUO (Fig. 4B). Most, but not all, tubular proliferation in the cortex on days 4 and 7 was restricted to dilated collecting ducts. Quantification of immunostaining shows

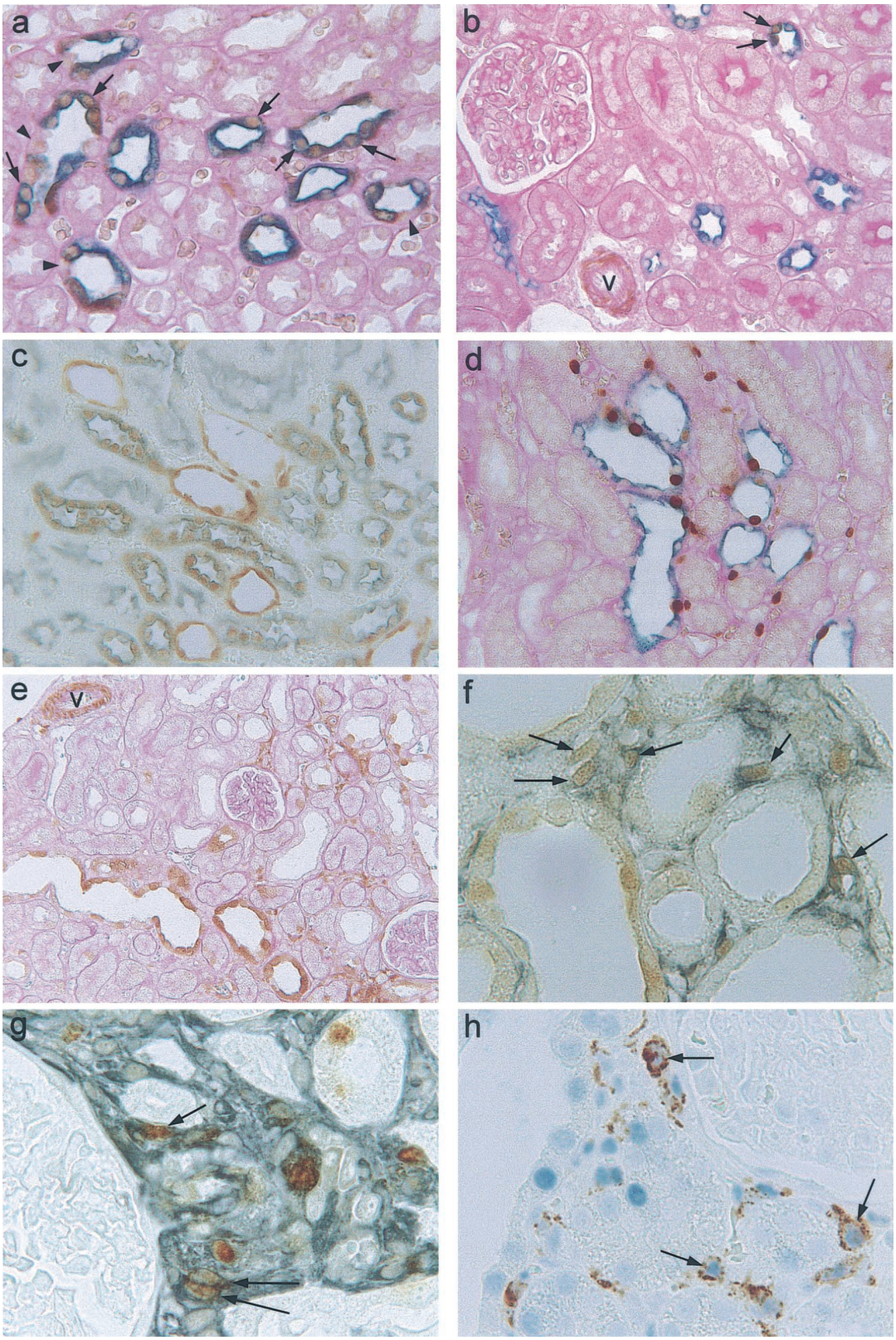

Fig. 3. Localization of extracellular signal-related kinase (ERK) activation and cell proliferation in normal and obstructed rat kidney using two-color immunohistochemistry. (*a*) Normal rat kidney shows co-localization of nuclear pERK (brown, arrows) and cytoplasmic aquaporin-2 (AQP-2) (blue) within collecting ducts in the outer medulla. Note that intercalating cells of the collecting ducts are negative for both pERK and AQP-2 (arrowheads). (*b*) Normal rat kidney cortex shows nuclear pERK staining (brown) in occasional cells within AQP-2+ collecting ducts (blue) (arrows show double-stained cells). Note also the pERK staining in the smooth muscle cells in a vessel wall (v). (*c*) Strong nuclear and

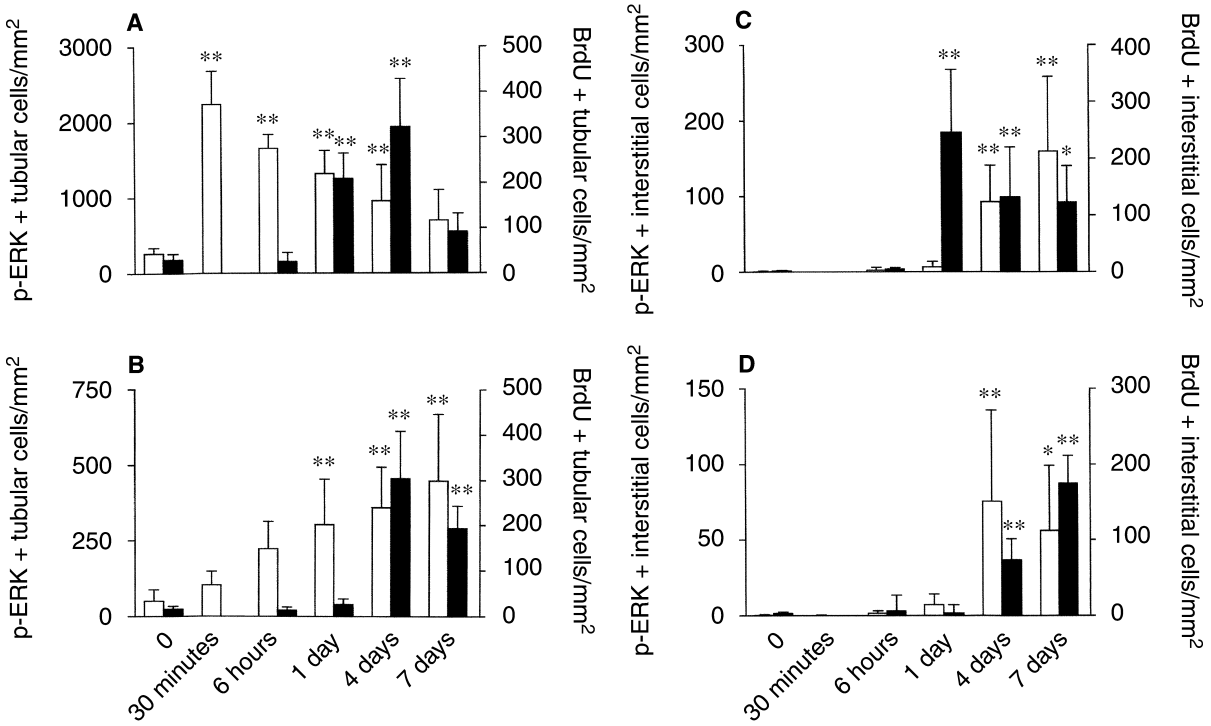

Fig. 4. Quantification of extracellular signal-regulated kinase (ERK) activation and cell proliferation in normal and obstructed rat kidney. Tissue sections from normal rat kidney and at different times after unilateral ureteric obstruction (UUO) were immunostained for pERK (□) or bromodeoxyuridine (BrdU) (■) and counterstained with periodic acid-Schiff (PAS) minus hematoxylin. The number of pERK and BrdU+ tubular cells per mm^2 are shown for the outer medulla (*A*), and the cortex (*B*). The number of pERK and BrdU+ interstitial cells per mm^2 are shown for the outer medulla (*C*), and the cortex (*D*). Data are expressed as mean ± SD for groups of six animals. $^*P < 0.05$; $^{**}P < 0.01$; versus normal by ANOVA (analysis of variance) with Dunnett multiple comparison post-test.

that ERK activation preceded tubular proliferation in both the outer medulla and cortex in the obstructed kidney (Fig. 4 A and B).

ERK activation in interstitial cells in the obstructed kidney

There was a significant increase in the number of outer medullary and cortical interstitial cells showing ERK activation on days 4 and 7 after obstruction (Fig. 4 C and D). This was coincident with the appearance of significant numbers of ED-1+ macrophages and α-SMA+ myofibroblasts in the interstitium (Fig. 5). Double immunohistochemistry staining identified ERK activation in numerous interstitial α-SMA+ myofibroblasts (Fig. 3f). However, only occasional ED-1+ macrophages exhibited ERK activation (not shown).

A significant proliferative response in the interstitial cells of the outer medulla was evident on day 1 after UUO (Fig. 4C), before significant macrophage or myofibroblast accumulation. Substantial proliferation of interstitial cells in the outer medulla and cortex was seen on days 4 and 7 after obstruction (Fig. 4C and D). Double immunostaining identified numerous interstitial BrdU + ED-1+ proliferating macrophages and BrdU + α-SMA+ proliferating myofibroblasts on days 4 and 7 (Fig. 3 g and h).

DISCUSSION

This study has localized ERK activation within the normal rat kidney and during renal damage caused by ureteric obstruction. The findings relating to normal kidney are discussed, followed by consideration of ERK activation and cell proliferation in the obstructed kidney.

cytoplasmic staining for pERK (brown) in both dilated collecting ducts and Tamm-Horsfall protein–positive thick ascending limb of Henle (TALH) (blue/gray) is shown 30 minutes after obstruction. (*d*) Day 1 after obstruction in the outer medulla shows bromodeoxyuridine (BrdU)+ proliferating cells (brown nuclei) in dilated AQP-2+ collecting duct (blue), TALH, and the interstitium. (*e*) Cortex on day 4 after obstruction shows strong pERK staining (brown) that is largely restricted to dilated collecting ducts and interstitial cells. (*f*) Cortex on day 4 after obstruction shows nuclear pERK staining (brown) in interstitial α-SMA+ myofibroblasts (blue/gray) (arrows). pERK staining is also evident in the dilated tubules. (*g*) Day 4 after obstruction shows BrdU+ nuclei (brown) in interstitial α-SMA+ myofibroblasts (blue/gray) (arrows). (*h*) Day 4 after obstruction showing BrdU+ nuclei (blue) in interstitial ED-1+ macrophages (brown, arrows). Panels (a), (b), (d), and (e) were counterstained with periodic acid-Schiff (PAS) minus hematoxylin. Original magnification, (a) to (d) ×400; (e) ×250; (f) to (h) ×1000.

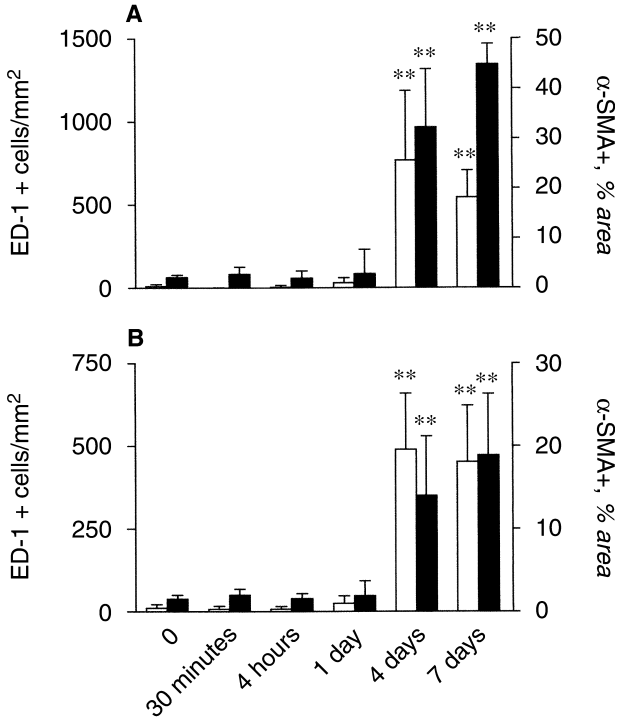

Fig. 5. Quantification of macrophage and myofibroblast accumulation in the obstructed kidney. Tissue sections from normal rat kidney and at different times after unilateral ureteric obstruction (UUO) were immunostained for ED-1+ macrophages (□) and α-smooth muscle actin (α-SMA)+ myofibroblasts (■). The number of ED-1+ macrophages per mm^2 and the percent of the tubulointerstitial area stained positive for α-SMA are shown for the outer medulla (*A*), and the cortex (*B*). Data are expressed as mean ± SD for groups of six animals. $^{**}P < 0.01$ versus normal by ANOVA (analysis of variance) with Dunnett multiple comparison post-test.

ERK activation in normal kidney

ERK-1 and ERK-2 were constitutively expressed throughout the tubular component of the nephron and collecting ducts, except for thin limbs of Henle. However, ERK activation was largely restricted to the collecting duct of the inner medulla. Double staining with AQP-2 indicated that ERK activation was restricted to the principal cells of the collecting duct. These results confirm and extend previous studies based upon examination of detergent extracts of kidney tissue which found that ERK activity was limited to the inner stripe of the medulla [7], with little or no ERK activity in whole cortex or isolated glomeruli in normal rat kidney [8]. Omori et al [12, 13] have used immunohistochemistry to localize pERK in human and rat fetal kidney. In adult rat kidney, Omori et al [13] described that pERK staining was restricted to distal tubules, although the staining pattern presented is very similar to that obtained in our current study. In addition to tubules, we identified ERK activation in vascular smooth muscle cells. This was not unique to the kidney as ERK activation was also seen vessels in spleen and lung.

What are the stimuli that induce ERK activation in the tubules of normal kidney? In vitro, hypertonic stress via urea and sodium chloride have been shown to activate ERK in a murine inner medullary collecting duct cell line [1, 14]. In vivo, hypertonic stress as a result of water restriction has been shown to activate ERK in the papilla [15]. In addition, in vitro studies have shown that ERK activation is important for hypertonic-induced transcription of AQP-5 in mouse lung epithelial cells [16]. Thus, the co-localization of ERK activation and AQP-2 expression supports the hypothesis that osmotic stress is a key regulator of ERK activation in normal renal physiology.

Tubular ERK activation in the obstructed kidney

Although there was a marked increase in tubular ERK activation in the obstructed kidney, the pattern of ERK activation was largely restricted to the dilated collecting ducts and TALH, with little ERK activation evident in convoluted tubules and no ERK activation seen in the glomerular tuft. There were two peaks of tubular ERK activation following ureteric obstruction. The early peak may be due to hypertonic stress following ureter ligation. Alternatively, the rapid ERK activation in the collecting duct and TALH in the medulla could operate via mechanical stretch due to fluid accumulation. Mechanical stretch has been shown to induce ERK activation in vascular smooth muscle cells [2], and in mesangial cells [6], but has yet to be demonstrated in tubular epithelial cells.

The later peak in tubular ERK activation over days 4 to 7 in the obstructed kidney may be due to continued mechanical stretch. Alternatively, tubular ERK activation may be part of the tubular proliferative response seen in this model. A mouse study found that renal hepatocyte growth factor (HGF) production is up-regulated following UUO and that HGF plays an important role in promoting tubular cell proliferation [17]. HGF has been shown to induce proliferation of the Madin-Darby canine kidney (MDCK) medullary collecting duct cell line [18], and a number of HGF-mediated effects are known to operate via the ERK pathway [19].

DNA synthesis in collecting ducts and distal tubules accompanies changes in ion transport activity in salt-loaded rats [20]. Therefore, it may be that the increase in tubular ERK activation after obstruction is linked to both proliferation and osmotic stress of ion transport in the obstructed kidney. Indeed, we observed ERK activation in both the nucleus and cytoplasm of collecting duct epithelial cells, which may participate in cell proliferation and ion transport, respectively.

ERK activation promotes tubular epithelial cell proliferation

Our study provides support for the hypothesis that ERK activation induces tubular epithelial cell proliferation in the injured kidney. Quantification of immunostaining showed a clear temporal relationship between

early tubular ERK activation and the subsequent tubular cell proliferation in first the medulla and then the cortex. In addition, ERK activation was largely restricted to parts of the tubule undergoing the proliferative response, dilated collecting ducts and TALH. Final proof of this hypothesis requires the use of specific ERK inhibitors in vivo. This may now be feasible following a recent study showing that administration of an ERK inhibitor can suppress mesangial cell proliferation in rat anti-Thy-1 nephritis [21].

ERK activation in interstitial cells in the obstructed kidney

ERK activation was also evident in interstitial cells in the obstructed kidney. Double staining identified that ERK activation was present in many interstitial α-SMA+ myofibroblasts, whereas very few ED-1+ macrophages exhibited ERK activation. This was an unexpected finding since proliferation of both of these cells types in vitro is ERK-dependent and there was substantial proliferation in both macrophages and myofibroblasts in the obstructed kidney. There are two possible reasons for this apparent contradiction. First, mitogenic stimuli can induce either a very transient (minutes) or a prolonged (hours) period of ERK activation in cultured cells [3, 22]. Thus, a transient ERK activation in macrophages may not be detected by immunostaining, whereas prolonged ERK activation in myofibroblasts is readily detectable. Alternatively, the prolonged duration of ERK activation in myofibroblasts may relate to other functions. For example, there is prolonged ERK activation in human fibroblasts during contraction of collagen matrices in vitro [23].

One other interesting observation in this study was the marked proliferative response of interstitial cells in the outer medulla on day 1 post-UUO. Since there were few macrophages or α-SMA+ myofibroblasts present at this time, we presume that this was a proliferative response of the resident renomedullary interstitial cells [9]. No ERK activation was detected in these interstitial cells prior to the proliferative response on day 1, suggesting that either proliferation of these cells can occur independently of ERK activation or that the transient nature of the ERK activation could not be detected.

The marked proliferative response of the resident renomedullary interstitial cells suggests that these cells may be precursors of the α-SMA+ myofibroblasts seen at later times in the outer medulla and cortex following ureteric obstruction. This is of interest since the origin of interstitial α-SMA+ myofibroblasts in renal fibrosis is an area of significant controversy, with different sources of myofibroblasts suggested, including migration of perivascular smooth muscle cells, blood-borne fibroblast precursors, and transdifferentiation of tubular epithelial cells [24, 25]. It may be that renomedullary interstitial cells need to be considered as an important source of interstitial myofibroblasts in the development of renal fibrosis.

CONCLUSION

In conclusion, this study has identified a discrete pattern of ERK activation in normal kidney and a marked increase in ERK activation in the obstructed kidney. The temporal and spatial relationship in which ERK activation precedes tubular cell proliferation suggests that ERK signaling plays a key role in tubular epithelial cell proliferation in the injured kidney.

ACKNOWLEDGMENTS

We thank Andreas Nelsbach of Cell Signaling Technology, Inc., for the phospho-ERK peptide. This study was funded by the National Health and Medical Research Council of Australia and the Australian Kidney Foundation.

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